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ANTIGENIC DIFFERENCES BETWEEN GONOCOCCI GROWN IN GUINEA PIG SKI--ETC(U)
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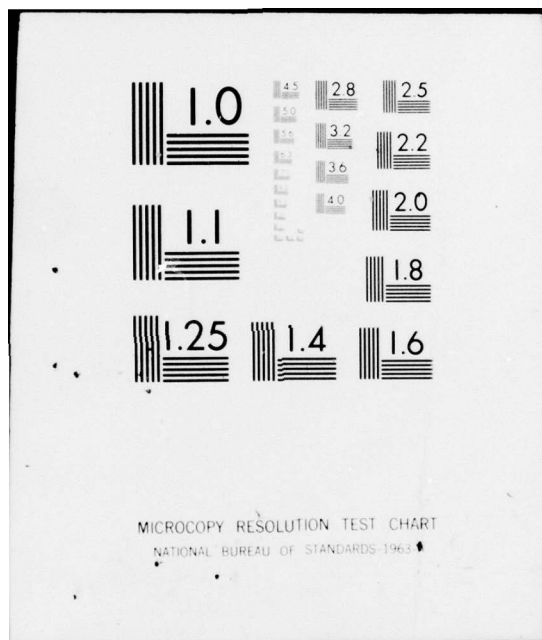


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REPORT NUMBER 1.

ANTIGENIC DIFFERENCES BETWEEN GONOCOCCI GROWN
IN GUINEA PIG SKIN CHAMBERS AND IN VITRO.

FINAL REPORT

PROFESSOR H. SMITH

1st October 1976

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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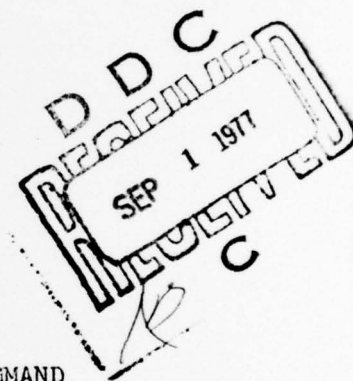
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SUMMARY

Efficient diagnostic tests are needed to reduce gonorrhoea. For many bacterial species antigens responsible for virulence revealed in organisms grown in vivo have been produced in vitro and used in diagnostic tests. Hence the objective was to examine gonococci grown in vivo for antigens not yet demonstrated in vitro. Urethral pus is not a suitable source of in vivo grown gonococci as relatively few organisms can be isolated from it. However in this pilot study (1 year) it was shown that gonococci grown in subcutaneous guinea pig chambers resembled urethral pus organisms and differed from the same strain grown in vitro. In particular, they resisted killing by human serum and phagocytes and showed in gel diffusion one or two antigens not produced by the parent strain in vitro. Two additional gonococcal isolates showed similar antigenic differences between organisms grown in guinea pigs and in vitro. These antigens revealed by growth in vivo might be important in designing future diagnostic tests.

FORWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

APPENDIXES

- 1) Morphological, biological and antigenic properties of Neisseria gonorrhoeae adapted to growth in guinea pig subcutaneous chambers.
C.W. Penn, D. Sen, D.R. Veale, K. Witt, N.J. Parsons and H. Smith,
1976, J.Gen.Microbiol. 97, 35-43.
- 2) Resistance of Neisseria gonorrhoeae grown in vivo to ingestion and digestion by phagocytes of human blood.
K. Witt, D.R. Veale, Hazel Finch, C.W. Penn D. Sen and H. Smith,
1976, J.Gen.Microbiol. 96, 341-350.

BODY OF REPORT

The problem was to see if gonococci grown in vivo formed antigens not produced by gonococci grown in vitro which might be of use in diagnostic tests for gonorrhoea.

BACKGROUND:

Gonorrhoea is the classic case of a disease, once controlled, rebounding under different social conditions. It is now pandemic and the main reasons appear to be sexual permissiveness, the high proportion of asymptotically infected females and antibiotic resistance (1, 2, 3, 4). Two public health requirements would help to control the disease. First the typing of gonococcal strains should be improved so that spread of particular infections can be investigated; and second diagnostic tests must be devised for detection of infection in asymptomatic carriers, (1, 2, 3, 4). An effective vaccine which would also help to control the disease may eventually be produced, but at present the outlook is not good. First, in humans, little or no lasting immunity to gonorrhoea follows natural infection (4). Then, the immunity against gonococcal infection of the genital tract of chimpanzees or of subcutaneous chambers implanted in guinea pigs, which develops after vaccination with large quantities of killed gonococci, is type specific (5, 6, 7).

Serological procedures would be a helpful adjunct to cultural and other methods that can be used for typing gonococcal strains; the useful antigens would be those showing type specificity such as appears to be the case for pili antigens (8). But serological procedures would be a greater boon to diagnosis since they could be used in surveys for asymptomatic carriers without the need for genital examination (3, 4, 9). Here the useful antigens would be those showing the greatest spread between different gonococcal strains without cross reactions with the antigens of commensal bacteria and other pathogens. The present serological tests (complement fixation, micro-flocculation of antigen coated particles of cholesterol and lecithin, micro-precipitin, haemagglutination and indirect fluorescent antibody techniques) using whole organisms, crude extracts of gonococci and purified endotoxin preparations, give positive reactions for 30-90% of patients with positive cultures and for about 10% of controls (3, 9). Clearly there is room for improvement. More sensitive techniques such as radioimmune assay might help but the cardinal requirement is a preparation of antigens which are both common and exclusive to gonococcal isolates.

For some other bacterial infections, the factors which determine the pathogenicity, especially the so-called aggressins (10) which inhibit host defence mechanisms in the early invasive stage of the disease, have formed the antigenic basis for diagnostic tests and typing procedures. Examples are, the O antigens of the enterobacteriaceae, the pneumococcal polysaccharides, the coagulase of staphylococci, the V and W antigens of Yersinia pestis and the toxins of Clostridium spp. In recent years some facets of the pathogenicity of gonococci have been elucidated but the antigens responsible are not clear.

Gonorrhoea is primarily a superficial infection of the lower urogenital tract with some penetration into the subepithelial layers (11). Electron microscopy on urethral scraping and on perfused fallopian tubes confirm the

adhesion of gonococci to and rapid infection of epithelial cells (12, 13). There is an acute inflammatory response (11). Undoubtedly this cellular response localises the infection in some cases, but gonococci can resist this response because infection can spread to other areas (e.g. fallopian tubes, prostate, joints) and sometimes generalises (1, 3, 4, 11, 14). Well staining extracellular and intracellular gonococci are seen in smears of urethral pus, suggesting that some gonococci might resist ingestion by phagocytes and others digestion. Also electron microscopy indicates some gonococci disintegrate in phagocytes but others look healthy and appear to be dividing (15). However, sequential observations on the same population were needed to prove intracellular survival and growth.

When Kellogg and his colleagues (16, 17) recognised stable virulent (types 1 and 2) and avirulent (types 3 and 4) types of gonococci the classical approach to mechanisms of pathogenicity could be made - comparisons of virulent and avirulent strains. The virulent types were pilated and the avirulent ones were not (18, 19). The virulent types resisted ingestion by human phagocytes more than the avirulent types (20-27). Pili may play a role in adherence to the urogenital tract (12, 13) but few pili can be seen on gonococci in urethral pus (15) and there was little difference in strength of adhesion to pieces of human endocervix between pilated and non pilated strain of gonococci (28). Whether pili are responsible for the resistance of virulent strains to phagocytosis is a matter of controversy (25, 26). Recently my colleagues and I have shown that a pilated strain of gonococcus resembling Kellogg type 2 survives and grows within human phagocytes (29, 30). The gonococcal components responsible for resistance to ingestion and digestion are unknown and may or may not be antigenic. They could be of use in diagnosis. Both virulent and avirulent strains produce in their cell walls a lipopolysaccharide endotoxin which appears similar to those of the enterobacteriaceae; however, its detailed chemistry and role in pathogenesis is not clear (1, 31). Antibodies to it may be involved in the bactericidal action on gonococci of human serum and complement (31) and it might also contribute to the cellular damage of the urogenital tract found in gonorrhoea.

APPROACH:

The behaviour of a microbe including its antigenic makeup is determined firstly by the genome and secondly by growth conditions which influence the extent of which the genetic information is expressed; changes of growth conditions produce different phenotypes from the same genome. For diagnosis we are interested in the antigens produced by bacteria when growing in the tissues of the infected host. When bacteria are removed from infected animals and grown in vitro the change in environment will induce phenotypes different from those found in vivo (32). In the past 17 years evidence has accumulated for many bacterial species (Bacillus anthracis, Yersinia pestis, staphylococci, Brucella abortus, Mycobacterium tuberculosis and streptococci) that organisms grown in infected animals are different chemically, antigenically and biologically from those grown in vitro; and these differences apply especially to the determinants of bacterial virulence (32, 33). Hence, although bacteria grown in vitro are convenient sources of antigens they may lack some antigens produced in vivo which could be important diagnostically. And a study of the behaviour of the organisms in vivo may resolve the hitherto unsolved problems of diagnosis of gonorrhoea. Methods are available for examining bacteria grown in vivo (32) and studies on such organisms have revealed antigenic determinants of virulence for B. anthracis, Y. pestis,

Br.abortus, staphylococci, V.cholerae (10, 32, 33).

Gonococci grown in vivo differ from those grown in vitro. Gonococci from urethral pus infected human volunteers more easily than gonococci grown in vitro (34), were more resistant to the killing action of human serum (34), had a different cell wall morphology (15, 35) and were less pilated (15). However, the examination of gonococci from urethral pus is difficult because of the small quantity (0.1-0.2 ml of pus containing $1-5 \times 10^6$ viable organisms per ml) that can be collected. Another source of gonococci grown in vivo became available when Arko (36) introduced infection of plastic chambers implanted subcutaneously in rabbits and steel springs in guinea pigs as models for research on gonococci. Using a modification of Arko's technique in guinea pigs my colleagues and I (37) showed that gonococci established persistent infection ($1-5 \times 10^6$ /ml of chamber fluid (4-5 ml) for more than 30 days) in the chambers despite the presence of an immediate and sustained inflammatory response which simulated the response in the human uro-genital tract. Strains of gonococci resembling the virulent (for man) Kellogg types 1 and 2 produced persistent infections, whereas a strain resembling the avirulent Kellogg type 4 did not. Finally, and important in relation to this proposal, three passages of the virulent strains through the chambers reduced the minimal infectious dose from 1000 to about 5 organisms, testifying to the increase in virulence effected by the in vivo conditions either by selection or phenotypic change. Hence, for this pilot study it was suggested that gonococci might be obtained from such guinea pig chambers in sufficient quantity for an examination of antigenic differences between them and gonococci grown in vitro in relation to any differences in biological properties that might be important in determining virulence.

RESULTS:

The detailed results are given in Appendixes 1 and 2, they are summarised as follows.

A pilated small colony forming strain (BS) of gonococci resembling Kellogg type 2 was passaged three times through guinea pig chambers and obtained regularly in sufficient quantities for comparison with the parent strain in serological, biological and other tests (App. 1 and 2).

The strain grown in vivo produced smaller colonies and was generally more sparsely pilated than the parent strain (BS) grown in vitro (App. 1)

The strain grown in vivo was more resistant than the parent strain grown in vitro to the killing action of fresh human serum and of human buffy coat phagocytes (App. 1 and 2).

Crude extracts of the surface components of the in vivo adapted strain, grown once on agar showed one or more protein components in polyacrylamide gel electrophoresis and produced in gel one or two immunoprecipitation lines against guinea pig antisera (evoked by infection of skin chambers) not found for corresponding extracts of the parent strain grown in vitro (App. 1).

Similar differences in immune precipitation in gels were obtained for two additional gonococcal isolates (AS and CS) in comparisons of extracts of the strains adapted to growth in guinea pig chambers and those of the parent strains grown in vitro. The experiments were the same as those described in App. 1 for strain BS and the same antisera were used. The

extracts of in vivo grown organisms from the two additional isolates produced one or two precipitation lines not formed by extracts of the in vitro grown strains. Furthermore, the additional immunoprecipitation lines formed by the two additional isolates when grown in guinea pig chambers corresponded with those formed by strain BS after growth in guinea pig chambers; thus adsorption of the serum with the latter organisms prevented the appearance of the lines formed by the extracts of the two additional in vivo grown isolates.

CONCLUSIONS:

In two properties, sparse piliation and resistance to fresh human serum, the organisms from guinea pig chambers simulated properties already reported for those from urethral pus (15, 34). In being more resistant to the killing actions of human serum and of human phagocytes the organisms grown in guinea pig chambers seemed more endowed with virulence determinants (i.e. aggressins) than those grown in vitro. And crude extracts of the surface components of the organisms adapted in vivo and grown once on agar, produced immunoprecipitation lines in gels and showed components in gel electrophoresis that were additional to those shown by corresponding extracts of in vitro grown organisms. Hence, the objectives of the pilot study had been achieved. Gonococci seem to produce in guinea pig chambers antigens which may be corrected with virulence (not only in guinea pig chambers but also in man, since human serum and phagocytes were used in the biological tests) - and these antigens are either not produced, or not produced to the same extent under normal cultural conditions in vitro.

RECOMMENDATIONS:

Extend these findings in three directions with the aim of identifying gonococcal antigens of potential use in diagnosis.

- 1) Survey many isolates of gonococci for additional antigens produced when they are grown in guinea pig chambers, and to see how far such additional antigens are common to the various isolates. The preliminary results on the two additional isolates (see above) are encouraging in this respect.
- 2) Extract and identify the antigens revealed by growth in guinea pig chambers; for this purpose attempts should be made to produce the antigens in quantity in vitro by modification of cultural conditions.
- 3) Survey the sera of patients with gonorrhoea for antibodies to the antigens revealed by growth of gonococci in guinea pig chambers.

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- 2) Witt, K., Veale, D.R., Finch, Hazel., Penn, C.W., Sen, D. and Smith, H. (1976) Resistance of Neisseria gonorrhoeae grown in vivo to ingestion and digestion by phagocytes of human blood. J.Gen.Microbiol. 96, 341-350.

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